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INDUCTION OF COLICINE SYNTHESIS BY MEANS OF MILLIMETER RADIATION

by

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For an institute of experimental and clinical oncology, Academy of Medical Sciences of DUSSOR and Scientific Research Institute of the Ministry of the Electronic Industry. (Submitted for publication March 17, 1971. Presented by a of the Academy of Medical Sciences of DUSSOR, M.M. Vlokhain.)

Abstract

The irradiation of the colicinogenic strain *E. coli* C. 600 (E_1) with electromagnetic waves in the millimeter range having a nonthermal intensity is capable of causing the induction of colicine synthesis. The dependence of the induction effect on wavelength, irradiation time and temperature of the test object is noted. (Bulletin of Experimental Biology, No. 4, p. 52, 1972).

A series of authors [1,3,5] have demonstrated that irradiation with millimeter waves having a nonthermal intensity may lead to perishing of a bacterial cell.

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The present work studies the action of waves in the millimeter range on the phenotypical expression of the genetic elements of bacteria which cause lethal synthesis.

Experimental Procedure

A colicogenic factor--The extra chromasomic genetic element of bacteria which causes lethal synthesis of the protein substrate colicine which exerts an antibacterial action relevant to bacteria of the same or related forms was chosen as the object of the investigation.

The investigations were carried out with the colicogenic strain E. coli C600 (E₁) and the E. coli K12S strain which is sensitive to the produced colicine and was obtained from the genetics and collection laboratory of the I.I. Mechnikov Institute.

The activity of colicine synthesis was determined by the method of Lacunae [15] in which a count is made of the individual bacteria which synthesize colicine. A two-hour culture of bacteria grown in Martin broth was irradiated in a Teflon vessel that was "transparent" to millimeter.

The oscillator producing the electromagnetic wave radiation consisted of a -wave tube of the OV612 type with an electron wave-tuning range extending from 5.7 to 8 nm and a maximum power in the continuous-wave mode that was close to 100 mW [4]. The irradiation was carried out from a horn antenna at wavelengths of 5.8, 6.15, 6.5, 6.57, and 7.1 nm. The power plus density amounted to 1 mW/cm².

The optimal dosages of culture and nutrient medium were selected which allowed reliable measurement of the transmitted power under the conditions requiring preservation of biological activity of the culture. The thickness of the bacterial suspension in the Teflon vessel amounted to 0.6 mm. The effect was estimated

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according to the magnitude of the induction coefficient:

$$K_i = \frac{N_{\ell. \text{ irr.}} / n_{\text{ce. irr.}}}{n_{\ell. \text{ nonirr.}} / n_{\text{ce. irr.}}}$$

Where $N_{\ell. \text{ irr.}}$ is the average number of lacunae in the irradiated culture; $n_{\ell. \text{ nonirr.}}$ is the average number of lacunae in the control for the same dilutions; $n_{\text{ce. irr.}}$ is the average number of irradiated cells; $n_{\text{ce. nonirr.}}$ is the average number of cells in the control for the same dilutions.

EXPERIMENTAL RESULTS

We have noted the dependence of the induction of lacunae formation by means of microwave irradiation on the irradiation time (see figure). The irradiation during the first thirty min did not affect the synthesis of colicine. Irradiation for a period of one hour caused an abrupt growth in the number of cells synthesizing colicine at the corresponding wavelengths. An increase in irradiation time to two hours caused a further growth of the induction coefficient. However, for a subsequent increase in irradiation time no clearly defined results were obtained.

Figure and Table RP53

Figure Caption: RP53, Dependence of the Induction Coefficient of Colicine Synthesis on Irradiation Time. The Irradiation Time is Plotted along the Axis of Abscissas (in hours); the Induction Coefficient is Plotted Along the Axis of Ordinates. 1) λ = 6.5 mm; 2) λ = 5.8 mm; 3) λ = 7.1 mm.

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Title of Table 1: The Induction Coefficient for the Synthesis of Colicine Under Microwave Irradiation.

Legend for Table 1: 1 - Indicator, 2 - Wavelengths (in mm), 3 - Number of Experiments, 4 - $K_{iav} \pm \delta K_i$ 5 - Index of Statistical Reliability, 6 - Error Probability (in %)

In the main the induction activity of the microwave radiation depended on the wavelength (see Table). Waves having a length of 5.8, 6.5 and 7.1 mm caused induction of colicine synthesis; wavelengths of 6.15 and 6.57 mm no substantial effect was observed. Statistical handling of the results obtained demonstrated their reliability which was likewise checked by a series of control experiments without irradiation. In this case the values of the induction coefficient remained constant and close to unity.

It is well-known that microwave irradiation may cause an increase in temperature in the irradiated object. Kohijaman Nomura [13] demonstrated the possibility of inducing colicine synthesis with an increase in temperature of the thermally sensitive mutant of the colicinogenic strain containing colicine E_2 . Although we used an *a fortiori* low irradiation intensities which did not exceed 1 mW/cm^2 at which an increase in temperature was improbable and the strain *E. coli* C600 (E_1) was not thermally sensitive, the indicated circumstances compelled us to monitor the temperature in the irradiated and nonirradiated systems. The temperature of the investigative system (the medium with the suspension of bacteria) was measured by means of a thermocouple. For a reading accuracy of 0.1 to 0.2° a temperature difference could not be successfully revealed.

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This allowed the conclusion to be drawn that the effect is of a nonthermal character.

In order to intensify the effect obtained irradiation was carried out in a thermostat at a temperature of 37°. In this case induction was also observed when irradiation lasted for 30 min. However, the effect could not be increased substantially.

Thus, it was demonstrated that irradiation with electromagnetic waves in the millimeter range is capable at specified wavelengths of inducing the synthesis of colicine in colicinogenic bacteria.

Heretofore the ability of various substances to induce colicine synthesis that is lethal for a colicinogenic cell has been associated mainly with their disintegration properties with respect to DNA or with their capability of blocking the synthesis of DNA. Ultraviolet radiation has such properties [17,10,12], and so do certain chemical preparations which disrupt the synthesis of DNA in a cell and are capable of causing damage to the DNA molecule [2,6,8,9,11,14]. The mechanism of induction by means of millimeter waves must be qualitatively different from the induction caused by these agents which are tropic with respect to DNA, since the energy of a millimeter-wave quantum is too small (10^4 times smaller than the energy than an ultraviolet-radiation quantum) to produce the rupture of any chemical bonds or to damage a DNA molecule. From this point of view millimeter radiation may be treated as a fundamentally new agent which without causing direct damage in a DNA molecule leads to disruption of the mechanism by which the function of the episomic genes are regulated in a cell.

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